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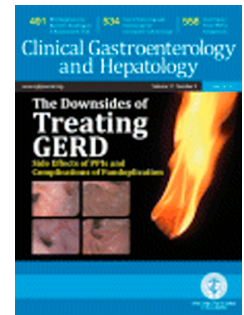
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Accepted Manuscript

Volatile Organic Compounds in Feces Associate With Response to Dietary Intervention in Patients With Irritable Bowel Syndrome

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Title: Volatile Organic Compounds in Feces Associate With Response to Dietary Intervention in Patients With Irritable Bowel Syndrome

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No conflict of interest to declare.

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Abstract:

Background & Aims: Dietary interventions are effective in management of patients with irritable bowel syndrome (IBS), although responses vary. We investigated whether fecal levels of volatile organic compounds (VOCs) associate with response to dietary interventions in patients with IBS.

Methods: Adults who fulfilled the Rome III criteria for IBS were recruited to a 2x2 factorial randomized controlled trial. Patients were randomly assigned to a group counselled to follow a diet low in fructans, galacto-oligosaccharides, lactose, fructose, and polyols (low-FODMAP diet, n=46) or a group that received placebo dietary advice (sham diet, n=47) for 4 weeks. Patients from each group were also given either a multi-strain probiotic or placebo supplement. Response was defined as a reduction of 50 points or more on the validated IBS symptom scoring system. Fecal samples were collected from participants at baseline and end of the 4-week study period; VOCs were analyzed by a gas-chromatography sensor device. VOC profiles were determined using a pipeline involving wavelet transformation followed by feature selection based on random forest. A partial least squares classifier was constructed to classify VOC profiles by response and were accuracies determined using 10-fold cross-validation.

Results: Data from 93 patients who completed the study (63 female) were used in the final analysis. More patients responded to the low-FODMAP diet (37/46, 80%) than the sham diet (21/47, 45%) ($P<.001$), but there was no difference in response between patients given the probiotic (31/49, 63%) vs the placebo (27/44, 61%) ($P=0.850$), with no interaction between the diet and supplement interventions. At baseline, VOC profiles contained 15 features that classified response to the low-FODMAP diet with an accuracy of 100% (95% CI, 96%–99%) and 10 features that classified response to probiotic with an accuracy of 89% (95% CI, 86%–92%). End of treatment models achieved similar predictive powers and accuracies.

Conclusion: Fecal VOC profiling is a low cost, non-invasive tool that might be used to predict responses of patients with IBS to LFD and probiotics and identify their mechanisms of action. ISRCTN registry no: 02275221

KEY WORDS: functional bowel disorder, low FODMAP diet; probiotics; microbiome

Introduction

Irritable bowel syndrome (IBS) is a chronic functional bowel disorder that is a major public health problem affecting 10-15% of adults worldwide.¹ The morbidity associated with IBS places a huge financial burden on both the healthcare system and workplace, and significantly impairs patients' quality of life.²

The pathogenesis of IBS is multifactorial, although the gut microbiota is thought to play a pivotal role.³ Some patients with IBS have intestinal dysbiosis characterised by a loss of microbial diversity, especially in those with diarrhoea-predominant IBS.⁴ Moreover, gut microbiota transplants from patients has been shown to induce an IBS-like disorder in mice⁵. The gut microbiota has also been used to predict IBS severity⁶. Nonetheless, the microbiota is currently an unrealistic diagnostic target or predictor of treatment outcome in practice because of the costs of its measurement.⁷

Evidence suggests that microbial function as opposed to composition may better predict clinical outcome.⁸ This is supported by research demonstrating that the faecal metabolome provides useful insight into IBS pathophysiology. More specifically, patterns of volatile organic compounds (VOC) were able to separate patients with IBS, inflammatory bowel disease and healthy controls with excellent accuracy.⁹ This research has been validated using both gas chromatography–mass spectrometry (GC-MS)¹⁰, considered the gold standard technique, and a new GC-sensor approach⁹. This validates the potential for VOC as a diagnostic biomarker for IBS and supports the contention that IBS is associated with abnormal microbial metabolism.

There is currently no universal treatment for IBS. Pharmaceutical and dietary intervention have some efficacy for the management of gastrointestinal symptoms, although the latter encourage self-management. Dietary restriction of fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) has been shown to significantly improve gastrointestinal symptoms in 50-80% of patients with IBS.^{11, 12} Nonetheless, the low FODMAP diet (LFD) has several limitations, including nutritional and microbiological sequelae and the labour intensive and complex nature of the diet.¹³ Probiotic supplementation has also shown some efficacy in IBS, although cost-to-benefit analysis limits widespread uptake.¹⁴

Personalised nutrition is an emerging concept whereby dietary intervention is based on a specific phenotypes (e.g. blood markers) or genotype (e.g. single nucleotide polymorphisms).¹⁵ Unsurprisingly, identifying an appropriate phenotypic and genotypic biomarker is pivotal to the success of personalised nutrition. Given the promising application of VOC as a diagnostic tool in IBS coupled with the fact that dietary interventions (LFD and probiotics) specifically target the gut microbiota, it is conceivable that faecal VOC may have a role in predicting response. Therefore, this exploratory study aimed to investigate whether a non-invasive diagnostic model using faecal VOC could be used to identify features associated with response to dietary intervention in IBS: (i) at baseline (i.e. predict response); and (ii) at end of treatment (i.e. explore potential mechanisms underpinning response).

Materials and methods

Our hypothesis was that specific VOC signatures at baseline would be able to predict response to LFD and probiotic interventions, and at follow-up would differ between responders and non-responders. In order to test these hypotheses, VOC profiles in baseline and end of treatment (4 weeks) faecal samples were analysed from a 2x2 factorial, multicentre, randomised, placebo-controlled trial of LFD and probiotics in the management of IBS.¹⁶

Clinical trial

The current analysis used clinical data and stool samples from a clinical trial, the methods of which are described in full elsewhere.¹⁶ Briefly, adults with diarrhoea-predominant (IBS-D), mixed subtype (IBS-M) or unsubtyped irritable bowel syndrome (IBS-U) according to Rome III criteria with no other major medical conditions were recruited from clinics at two hospitals in London, UK. Exclusion criteria were change to IBS medication, bowel preparation, and antibiotic therapy, prebiotics or probiotics during the previous four weeks. Research ethics committee approval for the trial and for VOC analysis was received from the London Fulham Research Ethics Committee (Reference 12/LO/1402) and patients gave informed consent prior to participation.

Patients were randomised to both diet (sham or LFD) and supplement (placebo or probiotic) groups, stratified by gender and diagnosis of IBS-D, for four weeks. Patients were masked to both diet and supplement allocations. The researcher who provided the dietary advice was not masked to diet allocation but was masked to supplement allocation. The placebo and probiotic supplements were identical in appearance, taste and presentation. Details of the dietary interventions are described in Supplementary material.

Symptoms were measured at baseline and follow-up using the IBS Symptom Scoring System (IBS-SSS).¹⁷ Patients with a change of <50 on the IBS-SSS were defined as non-responders. Further details of choice of clinical outcomes are described in Supplementary material. Differences in response rates between the diet and supplement groups were determined using Chi2 and differences in FODMAP intake between groups, using linear regression. Sensitivity analyses were undertaken to determine whether there was a significant interaction between the diet and supplement interventions. The null hypothesis was rejected at the 0.05 level. Statistical analyses of the clinical and dietary endpoints were performed using Stata (version 12, 2012, Statacorp, College Station, TX, USA).

Volatile organic compound analysis

At baseline and follow-up a whole fresh faecal sample was collected within one hour of passage, stored immediately on ice, homogenised in a stomacher machine and stored at -80°C until analysis.

Samples were shipped on dry ice to the Sensor Laboratory, where they were stored at -20°C. Subsequently, 750 mg aliquots were analysed by an in-house platform involving a gas-chromatography sensor device (Odoreader) and a computer pipeline for pattern recognition and sample classification.^{9, 18} A detailed description of the hardware and software used by the Odoreader has been published previously.¹⁸ Briefly, each sample was heated to 50°C for 10 minutes resulting in the release of VOCs from the faeces and their accumulation at the headspace of the vial. A total of 2 cm³ of its headspace were collected and injected into the GC column of the Odoreader.¹⁹ The 30 m SPB-1 sulphur GC column (Supelco, Sigma Aldrich) separates VOCs according to size and polarity. VOCs elute from the GC column and are

detected on a heated metal-oxide gas sensor controlled by an electronic circuit monitored by computer software. VOCs cause a change in electrical resistance of the circuit, which is recorded at 0.5 s intervals for 40 minutes per sample.

The resistance profiles produced by the Odoreader were analysed by an in-house-developed computer pipeline. The Odoreader platform was applied to detect patterns of VOCs able to predict response to: (i) LFD or probiotics using baseline samples; and (ii) LFD or probiotics using end of treatment samples. The pipeline is described in depth in Aggio et al. 2016.¹⁸ In summary, the Odoreader produces profiles of the sensor resistance vs. time, or chromatograms; the characteristics of the resistance vs time creates patterns that can be used to build models. The pattern is made up of a series of features. The pipeline performs chromatogram alignment, extracts wavelet coefficients and applies other data transformation techniques that improve the detection of volatile chemical patterns specific to each group, in this case responders and non-responders. Two random forest-based algorithms were applied to select the features that best describe the differences between responders and non-responders.^{17, 18} The selected features were submitted to Partial Least Squares (PLS) statistical modelling technique to classify unknown samples. Classification results were validated using 10-fold cross-validation repeated 15 times and principal component analysis (PCA) was applied to the transformed data.²⁰

The VOC extraction was undertaken in a blinded manner such that the samples were labeled using codes. The technician had no access to any clinical outcome data. For data analysis, the whole data analysis was automated and every step of the analysis was applied for all the samples, independent of class.

Statistical analyses were performed solely on the data processed by the Odoreader. Demographic or clinical features were not included in statistical modelling as our goal was to build a model that relied exclusively on the VOC. Confidence intervals (CI) were calculated using bootstrapping. Data analysis was carried out using R software.²¹

All authors had access to the study data and reviewed and approved the final manuscript.

Results

Clinical response

A total of 95 patients completed the study of whom 93 (63 females, 68%) provided sufficient faecal sample for analysis (n=86 from baseline visit and n=78 from follow-up). Of these 93 patients, 46 were randomised to LFD, 47 to sham diet, and 49 to probiotic, 44 to placebo as follows: LFD/probiotic (n=26), LFD/placebo (n=20), sham/probiotic (n=23) and sham/placebo (n=24) (**Table 1**). All 93 patients were classified as compliant with diet (sham, LFD), whereas 86 were classified as compliant with the supplement (three to probiotic, four to placebo). All patients were included in this analysis. At the end of the study, those randomised to the LFD had a significantly lower total FODMAP intake (20 ± 8 g/day) compared with sham diet (33 ± 16 g/day) ($p < 0.001$).

There was no interaction between the interventions (diet/supplement) for the clinical endpoint (IBS-SSS) ($p = 0.425$) and therefore results are presented separately for diet (LFD vs sham) and supplement (probiotic vs placebo). More patients responded to LFD (37/46, 80%) compared with sham (21/47, 45%) ($p < 0.001$), with a mean change in IBS-SSS score of -130 ± 81 and -49 ± 75 ($p < 0.001$), respectively. There was no difference in the numbers of responders between probiotic (31/49, 63%) and placebo (27/44, 61%) ($p = 0.850$) and no difference in the mean change in IBS-SSS score between probiotic (-88 ± 78) and placebo (-90 ± 98) ($p = 0.921$).

Volatile organic compound modelling

The VOC patterns showed clear separation between responders and non-responders to both LFD and probiotic at both baseline and end of treatment. The PCAs identified: (i) 15 compounds at baseline that explained 25% of variation in response to LFD, and 10 compounds that explained 30% of variation in response to probiotic; and (ii) 9 compounds at end of treatment that explained 31% of response to LFD and 11 compounds that explained 27% of variation in response to the probiotic (Figure 1).

The PLS model to classify responders and non-responders on baseline samples showed very high accuracy for both interventions. The PLS model from the LFD group predicted response

to the diet with median accuracy, sensitivity and specificity of 100% (**Table 2**). However, the same LFD baseline PLS model showed low accuracy when classifying baseline samples from the patients in the control group (sham/placebo) (**Table 3**), indicating that the model was specific to response to the LFD/ probiotic rather than merely response *per se*.

Similarly, the PLS model on baseline samples from the probiotic group predicted response to probiotic with median accuracy of 89%, median sensitivity of 100% and median specificity of 75% (**Table 2**). Again, the probiotic baseline PLS model showed low accuracy when classifying baseline samples from the control group (**Table 3**).

The PLS models on end of treatment samples also showed high accuracy for both interventions. The PLS model from the low FODMAP group predicted response to the diet with median accuracy, sensitivity and specificity of 100% and the model from the probiotic group predicted response with a median accuracy of 89% and sensitivity and specificity of 100% (**Supplementary Table**).

Although matching unique features to individual compounds is outside the scope of the Odoreader platform, one of the selected features from the end of treatment samples was shared across predicting response to both the LFD and probiotic intervention.

Discussion

Dietary intervention can be an effective management strategy for IBS, but little is known regarding who will, and will not, respond. This study measured VOC in baseline fecal samples, and through building separate models using key features of VOC profiles could correctly predict response to the LFD in 100% and to probiotic in 89%, thus potentially being a low cost, non-invasive tool that may pave the way for personalised treatment in IBS.

To our knowledge, only three studies have investigated predictors of response to the LFD in IBS, and all involve analysis of the microbiome or their metabolites. The first was a 2-day cross-over, feeding trial in children showing that, at baseline, responders were enriched in *Bacteroides*, *Ruminococcaceae* and *Faecalibacterium prausnitzii*, all known for saccharolytic metabolic capacity.²² A more recent randomised controlled trial (RCT) comparing the LFD

with standard dietary advice²³, showed that responders to a LFD could be predicted based upon the baseline level of deviation from a normobiosis using a commercially available test.²⁴ Finally, a clinical evaluation reported that predictive factors of response were chronic diarrhoea and peak breath methane concentrations.²⁵ In terms of probiotics, despite the greater numbers of RCTs in IBS (compared with LFD)^{14, 26}, this is the first study to investigate predictors of response to probiotic in IBS.

There are challenges in comparing findings from these previous studies with our own. Firstly, these studies included different potential markers, and therefore on completion, the resulting predictive model contained vastly different biomarkers of response. Secondly, response has been defined differently in the various studies, including meeting the minimally clinically important difference on the IBS-SSS²³ of >50 points (as used here), a >50% reduction in abdominal pain frequency²² and adequate symptom relief²⁵. Nonetheless, the current study is consistent with identifying microbiota and their metabolites as important in predicting response. The limitation of previously identified biomarkers include cost and time burden associated with microbiome analysis and the lack of standardised pre-test preparation and methodology associated with breath testing. Although VOC profiling is a relatively new technique, it may have numerous advantages over directly measuring the microbiome or single metabolites, as once a sampling protocol is developed it could be analysed in centre and be widely and rapidly available at one fifth of the current cost of microbial sequencing techniques.

Clinical trials indicate that the LFD is effective in 50-80% of patients. However, predicting response is clinically important because the diet requires intensive dietary counselling and impacts on both the gut microbiome and nutrient intake¹¹, and therefore avoiding this intervention in the 20-50% who are unlikely to respond would be an important clinical advance. In contrast, probiotics are widely accessible, comparatively easy to adhere to and have no known impact on nutrient intake and therefore pose less of a burden than a LFD. Nonetheless, the negative connotations associated with a failed therapy, such as loss of patient trust, in addition to the costs associated with unnecessary probiotic use reinforces the value of a biomarker that predicts response across both interventions.

There are several possible explanations why VOC patterns could predict response to the LFD. Many VOCs are created from bacterial metabolism of indigestible food substrates that reach the colon, representing both microbial metabolic activity and diet.^{27, 28} Therefore VOC patterns at baseline may reflect the (patho)physiology of an individual's IBS. For instance, eating a 'normal' diet, naturally high in FODMAPs, in the presence of IBS-associated dysbiosis may generate specific products of fermentation that give rise to symptoms. Once the 'normal' diet has been replaced by a LFD, there is less substrate for bacterial metabolism: consequently gas production (and the associated symptoms) are reduced.²⁹ Based on this hypothesis, only people with the sacchrolytic-rich, IBS-associated dysbiosis eating a high FODMAP diet may exhibit the specific VOC pattern predictive of response.

Similarly, with the probiotic intervention, only patients whose symptoms are triggered by IBS-associated dysbiosis in theory may respond to the probiotic intervention. Interestingly, microbial dysbiosis has been shown in approximately 60% of IBS cases³⁰ which reflects the probiotic response rate observed in this study. Nonetheless, validating these models in an independent sample is warranted to confirm the predictive potential of baseline VOC patterns. The probiotic model built in the current study was based upon response to a multi-strain probiotic formulation. Probiotics vary in their microbiological characteristics, and their physiological and clinical impact in IBS and it is therefore unknown whether the same model would predict response to a different probiotic.

In addition to the predictive nature of the baseline VOC patterns, the end of treatment models may provide additional insight into the pathophysiology of symptom generation and mechanisms underpinning dietary intervention. This study identified unique VOC patterns at follow-up that maximally separated responders and non-responders. This suggests a divergence in microbial metabolic activity between those who experience success or failure with the intervention. The reason for the divergence may reflect either dietary compliance or true inter-individual variations that may relate to the underlying drivers of IBS, such as dysbiosis. Irrespective, investigating the key features within the VOC profile may hold important clues into the mechanism of action underpinning respective dietary interventions. One of the predictive features identified in this study was shared across responders to both the LFD and probiotic, suggesting some commonalities in mechanisms.

Identifying the features is outside the scope and capability of the Odoreader. Ongoing research is underway using gas-chromatography mass-spectrometry to identify the features that both predict and result in a clinical response.

Despite the promising nature of this research, several limitations are worthy of consideration. Firstly, its novel nature means there was no data to inform a power calculation and it should be viewed as an exploratory study with the data generated used to inform future external validation studies powered to detect differences in the features identified here. Secondly, while the predictive models demonstrated high accuracy, specificity and sensitivity, external validation in a new, larger cohort of IBS patients is needed to determine the clinical validity of this work. Thirdly, the purpose of the Odoreader is to identify patterns of VOCs, not individual VOCs. While this limitation enables the tool to be time and cost-efficient, it limits detailed investigation of the potential mechanisms linked to individual VOCs. Finally, it is feasible that the 2x2 factorial design utilizing two interventions (LFD, probiotics, both, neither) may have clouded the results. Nonetheless, there was no interaction between interventions, meaning they did not have a synergistic or antagonistic effect on clinical response. Further, it is common in the clinical setting for patients to follow multiple interventions (e.g. LFD and probiotics), therefore the fact that VOC profile could predict response to one intervention despite some patients also receiving an additional intervention is testament to the potential clinical utility.

In summary, this exploratory study suggests that fecal VOC profiling is a promising non-invasive tool that may not only help predict response to dietary interventions but opens up a new opportunity to better understand the pathophysiology of IBS and mechanisms underpinning therapeutic response. This low-cost tool has the potential to help advance the clinical management of IBS paving the way forward for personalised nutrition

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TABLES

Table 1: Baseline characteristics of participants by treatment arm and by treatment response

Baseline Characteristics	Low FODMAP diet + Probiotic (n=26)	FODMAP + Placebo (n=20)	Sham + Probiotic (n=23)	Sham + Placebo (n=24)	Low FODMAP diet		Probiotic	
					Responders (n=37)	Non responder (n=9)	Responders (n=31)	Non responder (n=18)
Age (years)	38 (12)	35(11)	36 (12)	32 (12)	37 (13)	32 (12)	38 (13)	35 (11)
Female	17 (65)	16 (80)	15 (65)	15 (63)	26 (70)	7 (78)	21 (68)	11 (61)
Symptom duration (m)	106 (133)	64 (84)	76 (99)	49 (53)	82 (107)	111 (149)	89 (116)	97 (125)
IBS subtype								
IBS-D	19 (73)	12 (60)	16 (67)	16 (67)	23 (62)	8 (89)	19 (61)	15 (83)
IBS-M	6 (23)	5 (25)	5 (21)	5 (21)	10 (27)	1 (11)	9 (29)	2 (11)
IBS-U	1 (4)	3 (15)	3 (13)	3 (13)	4 (11)	0 (0)	3 (10)	1 (6)
Ethnicity (white)	21 (81)	17 (85)	20 (87)	19 (79)	32 (86)	6 (67)	25 (81)	16 (89)
Smoker	5 (19)	0 (0)	1 (4)	1 (4)	2 (5)	3 (33)	3 (10)	4 (22)
Vegetarian	1 (4)	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)	1 (3)	1 (6)
Weight (kg)	68 (14)	68 (11)	76 (23)	72 (15)	68 (13)	69 (13)	71 (18)	72 (21)
BMI (kg/m ²)	24 (4)	25 (4)	26 (6)	25 (5)	24 (4)	25 (4)	25 (5)	24 (5)
IBS-SSS total score	285 (75)	313 (74)	269 (78)	268 (72)	298 (76)	293 (76)	292 (81)	253 (60)
FODMAP intake (g/day)	33 (14)	27 (9)	32 (16)	33 (11)	32 (12)	25 (12)	32 (13)	34 (17)

Data presented as mean (SD) for continuous data and frequency (%) for dichotomous data.

BMI, body mass index; IBS-D, diarrhoea-predominant IBS; IBS-M, mixed subtype IBS; IBS-U, unsubtyped IBS; IBS-SSS, Irritable Bowel Severity Scoring System

Table 2: Accuracy, sensitivity and specificity of the baseline low FODMAP model and baseline probiotic model in predicting response to the low FODMAP diet or probiotic intervention or sham/placebo

Baseline Model	Responders	Non-responders	Accuracy (%)			Sensitivity (%)			Specificity (%)		
			Median	Mean	C.I.	Median	Mean	C.I.	Median	Mean	C.I.
Low FODMAP diet (n=44)	35	9	100	97	[96-99]	100	100	[100-100]	100	88	[80-94]
Probiotic (n=45)	29	16	89	89	[86-92]	100	93	[90-96]	75	82	[75-97]

Results of 10-fold cross-validation produced by the Odoreader platform when classifying baseline samples from patients who went on to respond and not-respond to low FODMAP or probiotic interventions. Partial least squares was used as the modelling technique. 95% C.I. = confidence intervals

Table 3 Accuracy, sensitivity and specificity of the baseline model in predicting response in the control group

	Control group (sham/placebo), n=22		Accuracy	Sensitivity	Specificity
	Responders	Non-Responders			
Low FODMAP diet model (baseline)			9/22 (40.9%)	5/8 (62.5%)	4/14 (28.6%)
Responders	5	10			
Non-responders	3	4			
Probiotic model (baseline)			10/22 (45.5%)	6/8 (75%)	4/14 (28.6%)
Responders	6	10			
Non-responders	2	4			

Figure 1. Principal component analysis of faecal samples from patients responding and not responding to low FODMAP diet or probiotic before and after intervention.

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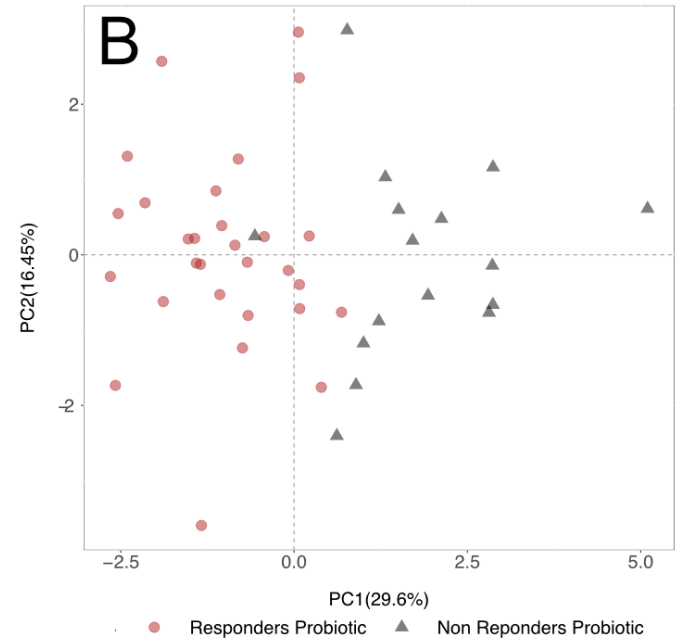
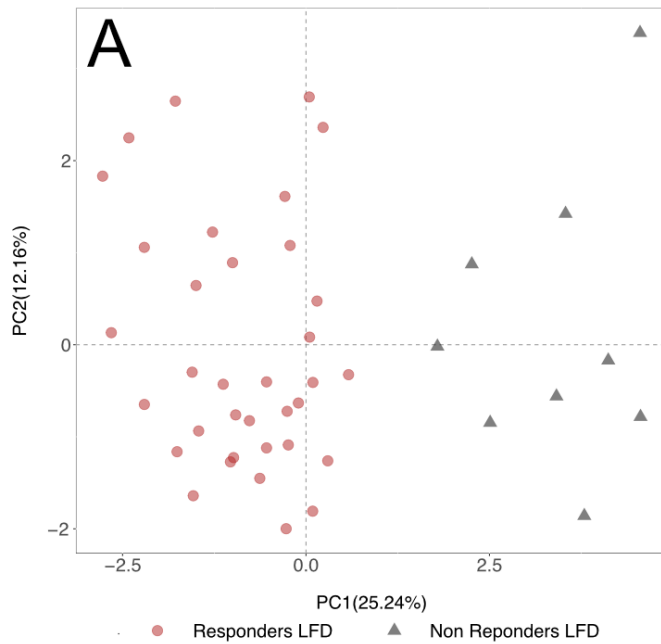
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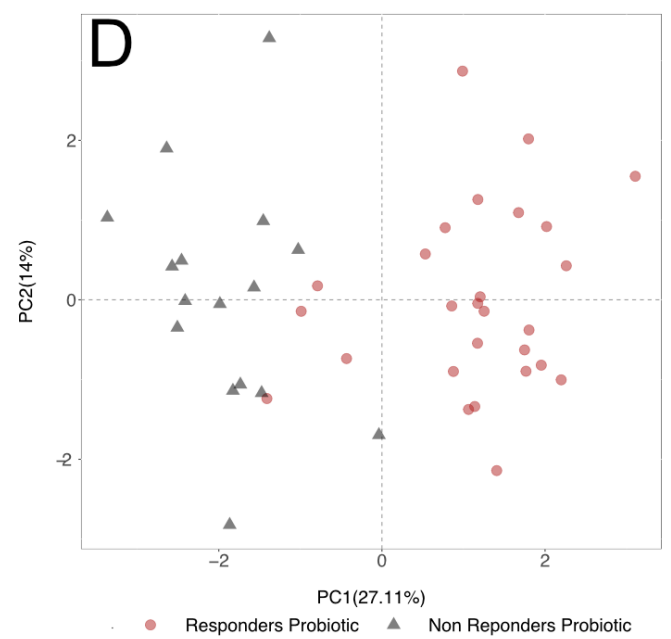
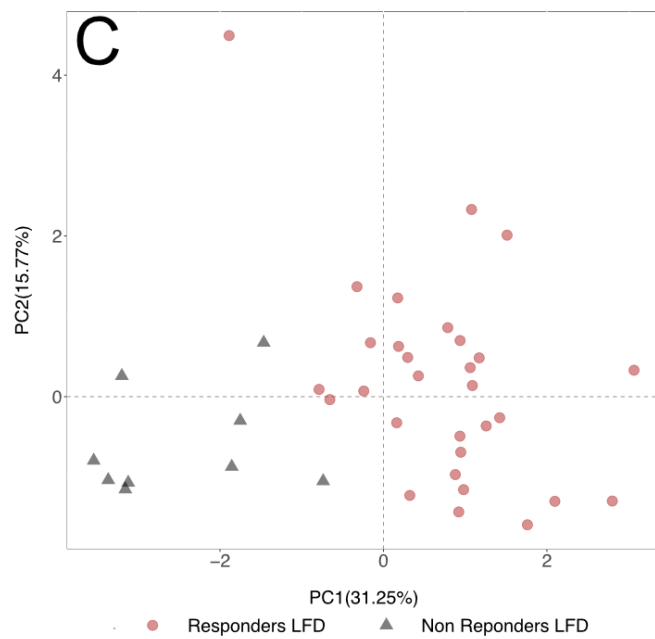
Low FODMAP Diet (LFD)

Probiotic

Baseline



End of treatment



Supplementary data

Dietary intervention

The LFD involves restricting dietary intake of fructans, galacto-oligosaccharides (GOS), lactose, fructose in excess of glucose, and polyols, and is described elsewhere.¹ The sham diet was designed for this trial and restricted a similar number of staple and non-staple foods, required a similar intensity and duration of dietary counselling and similar difficulty of dietary change to the LFD and did not impact on intakes of nutrients, fibre and FODMAPs.² Dietary compliance was measured weekly by self-report during weekly telephone calls. Patients were considered compliant if they reported following the diet $\geq 50\%$ of the time on at least two of the four weekly assessments in line with previous work.³

The probiotic was a multi-strain preparation containing *Streptococcus thermophilus* DSM 24731, *Bifidobacterium breve* DSM 24732, *B. longum* DSM 24736, *B. infantis* DSM 24737, *Lactobacillus acidophilus* DSM 24735, *L. plantarum* DSM 24730, *L. paracasei* DSM 24733, *L. delbrueckii* subsp. *bulgaricus* DSM 24734 (now exclusively available in Europe under the trademark Vivomixx® and in the United States under the trademark Visbiome™) and was provided in sachets in freeze dried form with maltose and silicon dioxide as inactive excipients. The placebo sachets contained the same inactive excipients but no bacteria. Participants received two sachets per day (11-95 log₁₀ bacteria in the intervention group) to be taken in the morning with cold food or fluid. Patients were considered compliant with the supplement if 80% of sachets were taken based on return of all unused sachets.

Clinical outcomes

Symptoms were measured at baseline and follow-up using the IBS Symptom Scoring System (IBS-SSS).⁴ The primary outcome of the parent study used the global symptom questionnaire to define response, which requires synthesis of the totality of patient symptoms into a single binary response.⁵ However, given the focus of this predictive work was the clinical setting, the IBS-SSS multi-item instrument was deemed to be a more clinically meaningful outcome to measure in the current study as it combines four individual components: abdominal pain, distension, bowel habit and interference with life. In addition, a reduction from baseline of ≥ 50 points on the IBS-SSS is widely accepted as a minimally clinically important difference (MCID) to define patients as responders.⁴ Patients with a change of < 50 on the IBS-SSS were defined as non-responders.

SUPPLEMENTARY TABLE: Accuracy, sensitivity and specificity of the end of treatment low FODMAP diet model and end of treatment probiotic model in allocating responders and non-responders to the low FODMAP diet or probiotic intervention or sham/placebo

End of Treatment Model	Responders	Non-responders	Accuracy (%)			Sensitivity (%)			Specificity (%)		
			Median	Mean	C.I.	Median	Mean	C.I.	Median	Mean	C.I.
Low FODMAP diet(n=39)	30	9	100	96	[93-98]	100	100	[99-100]	100	82	[70-92]
Probiotic (n=44)	29	16	89	91	[88-94]	100	92	[88-96]	100	90	[84-96]

Results of 10-fold cross-validation produced by the Odoreader platform when classifying baseline samples from patients who went on to respond and not respond to low FODMAP diet or probiotic interventions. Partial least squares was used as the modelling technique. 95% C.I. = confidence intervals

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